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COMPLETE SPECIFICATION

STABILIZATION OF 13,14-DIHYDRO-15-KETOPROSTAGLANDINS

We, KABUSHIKIKAISHA UENO SEIYAKU OYO KENKYUJO, a corporation of Japan of 4-8, 2-chome, Koraibashi, Chuo-ku, Osaka-shi, Osaka-fu, Japan,

do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:-

228111

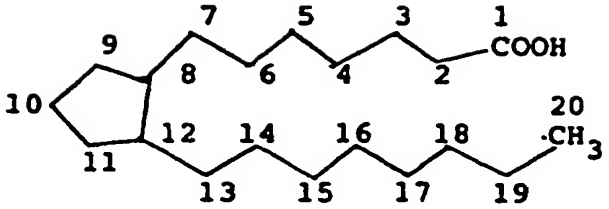
STABILIZATION OF 13,14-DIHYDRO-15-KETOPROSTAGLANDINS

BACKGROUND OF THE INVENTION

Field of the Invention

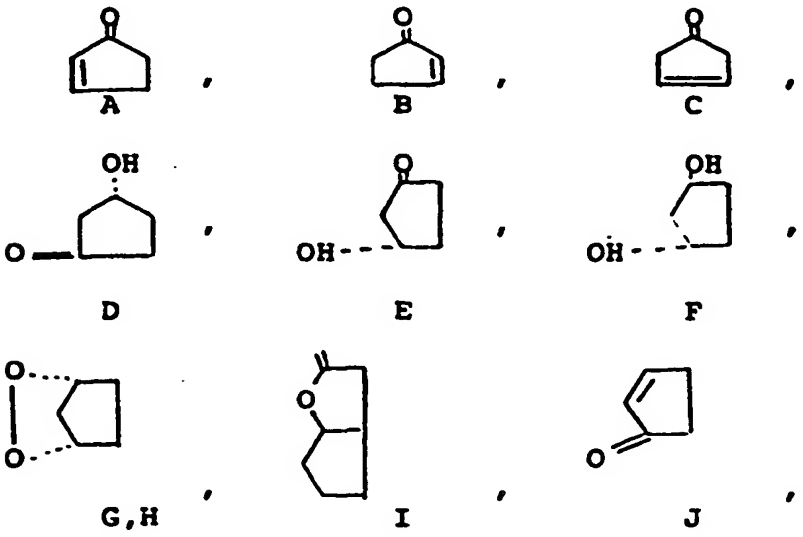
The present invention relates to the stabilization of 13,14-dihydro-15-ketoprostaglandins, which find various applications in the medical field.

Prostaglandins (hereinafter, referred to as PG) are members of a class of organic carboxylic acids that are contained in human and most other mammalian tissues or organs and that exhibit a wide range of physiological activities. Naturally occurring prostagladins possess as a common structural feature the prostanoic acid skeleton:



(A)

while some of synthetic analogues have somewhat modified skeletons. The natural prostaglandins are classified based on the structual feature of five-membered cycle moiety as:



and also on the presence or absence of unsaturation and/or oxidation in the chain moiety as:

Subscript 1 - - - 15-OH

Subscript 2 - - - 5,6-unsaturated-15-OH

Subscript 3 - - - 5,6- and 17,18-diunsaturated-15-OH.

All the natural prostaglandins are 13,14-unsaturated.

However, the presence of some 13,14-saturated prostaglandins, such as 13,14-dihydro-15-keto-PGD_2, 13,14-dihydro-15-keto-PGE_2 and 13, 14-dihydro-15-keto-$PGF_{2\alpha}$, are known as the metabolites which have been believed to have no physiological activity.

Background Information

While the prostaglandins have a wide range of physiological activities and are useful as medicaments based on their respective activities, they have a common fault that they are generally unstable. Various attempts have been made to improve the stability of prostaglandins.

For example, formation of inclusion compounds of PGs or alkyl ester thereof with cyclodextrin (hereinafter, referred to as CD) was disclosed in Japanese Patent Publication No. 3362/1975. Injectable preparations obtained by lyophilyzing PGs or analogues thereof and CD was disclosed in Japanese Patent Publication No. 43569/1979. Injectable preparation obtained by lyophilizing PGE or analogues thereof, CD and ascorbic acid or citric acid was disclosed in Japanese Patent Publication No. 43570/1979. Formation of inclusion compounds of $PGF_{2\alpha}$ analogues with CD was disclosed in Japanese Patent Publication No. 24369/1986. Etherized CDs could be used in the same manner as CD itself for stabilizing PGE as disclosed in Japanese Patent Publication (unexamined) No. 10525/1984.

The inventors discovered that the 13,14-dihydro-15-keto-PGs have certain physiological activities, contrary to the traditional knowledge that they have no such activities (EP-A 0,284,180, EP-A 0,281,239, EP-A 0,289,349 and EP-A 0,292,177). Attempts have been made, in turn, to stabilize the 13,14-dihydro-15-keto-PGs by means of CD, which, however, have come out to be a failure, confirming that they cannot be stabilized by CD as the result of repeated experiments (unpublished). This means that the stabilization of 13,14-saturated-15-keto-PGs cannot be predicted from the stabilization of 13,14-unsaturated-15-keto-PGs.

SUMMARY OF THE INVENTION

In the first aspect, the present invention provides a stabilized 13,14-dihydro-15-ketoprostaglandin composition comprising an intimate mixture of

a) a therapeutically effective amount of at least one compound selected from 13,14-dihydro-15-ketoprostaglandins and

b) at least one compound selected from pharmaceutically acceptable etherized cyclodextrins.

In the second aspect, the present invention provides a method of preparing a stabilized 13,14-dihydro-15- ketoprostaglandin composition which comprises intimately mixing

a) a therapeutically effective amount of at least one compound selected from 13,14-dihydro-15-ketoprostaglandins, and

b) at least one compound selected from pharmaceutically acceptable etherized cyclodextrins.

In the third aspect, the present invention provides a method of stablizing 13,14-dihydro-15-ketoprostaglandins which comprises contacting (A) 13,14-dihydro-15-ketoprostaglandin with (B) etherized cyclodextrin in a solvent capable of at least partly dissolving at least one of (A) and (B).

In the fourth aspect, the present invention provides a stabilizer for 13,14-dihydro-15-ketoprostaglandins comprising etherized cyclodextrin.

According to the invention, it has now be discovered that etherized CDs are useful for stabilization and solubilization of 13,14-dihydro-15-keto-PGs, after exhausting testing of various compounds. It is very surprising that etherized CDs are helpful in the case where CD is not helpful, in view of the close similarity of chemical structure between etherized and unetherized cyclodextrins. Etherized CDs appear to form adducts with 13,14-dihydro-15- keto-PGs and are presumed to form inclusion compounds analogously to CD.

DETAILED DESCRIPTION OF THE INVENTION

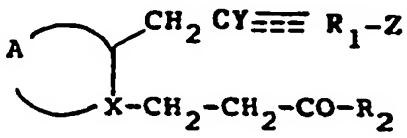
Nomenclature

Nomenclature of 13,14-dihydro-15-keto-PGs herein uses the numbering system of prostanoic acid represented in the formula (A) shown above.

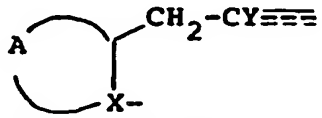
While the formula (A) shows a basic skeleton having twenty carbon atoms, the 13,14-dihydro-15-keto-PGs used in the invention are not limited to those having the same number of carbon atoms. The carbon atoms in the Formula (A) are numbered 2 to 7 on the alpha-chain starting from the alpha-carbon adjacent to the carboxylic carbon which is numbered 1 and towards the five-membered ring, 8 to 12 on the said ring starting from the carbon on which the alpha-chain is attached, and 13 to 20 on the omega-chain starting from the carbon adjacent to the ring. When number of carbon atoms is decreased in the alpha-chain, the number is deleted in order starting from 2-position and when number of carbon atoms is increased in the alpha-chain, compounds are named as substituted derivatives having respective substituents at 1-position in place of carboxyl group (C-1).

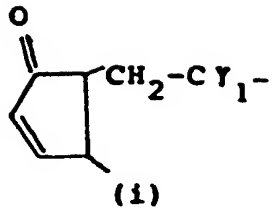
Preferred Embodiments

Within the 13,14-dihydro-15-ketoprostaglandins as described above, preferred compounds are those represented by the formula(I):

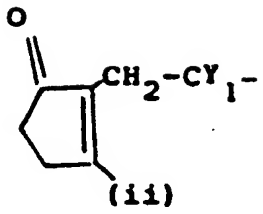


(I)

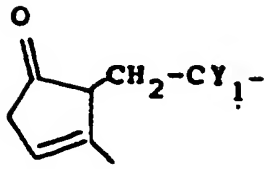
wherein the group:  is a radical selected from the group consisting of the following formulae:



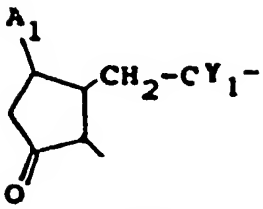
(i)



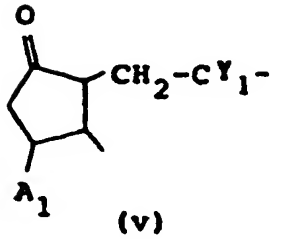
(ii)



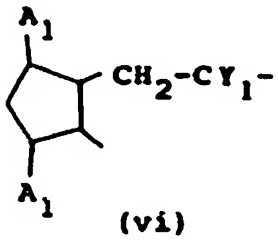
(iii)



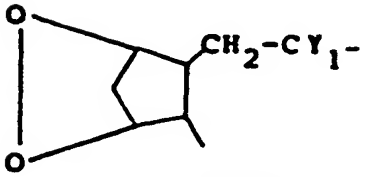
(iv)



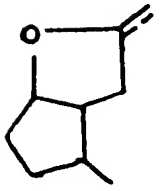
(v)



(vi)

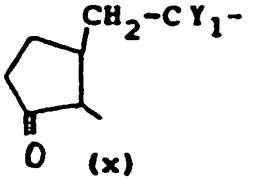


(vii)

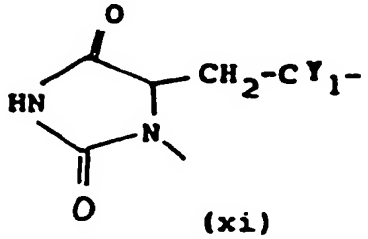


(viii)

(ix)



(x)



(xi)

A_1 is hydroxy, lower alkyl or hydroxy(lower)alkyl,

Y_1 is 0, 1 or 2 hydrogen atoms or oxo,

Z is hydroxymethylcarbonyl, carboxy or a functional derivative of carboxy,

R_1 is saturated or unsaturated lower aliphatic hydrocarbon residue,

R_2 is saturated or unsaturated lower aliphatic hydrocarbon residue which is unsubstituted or substituted with at least one substituent selected from the group consisting of hydroxy, halo, lower-alkoxyphenyl and phenoxy,

the symbol of a line and a dotted line is single bond or double bond, and the symbol of a line and two dotted line is single bond, double bond or triple bond, or a pharmaceutically acceptable salt thereof.

Definitions

As used herein, the term "lower" is intended to include a group having 1 to 6 carbon atoms unless otherwise specified.

The term "lower" as a group or a moiety in hydroxy(lower)alkyl includes saturated and straight or branched chain hydrocarbon radicals containing 1 to 6, preferably 1 to 5 and more preferably 1 to 4 carbon atoms, such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, t-butyl, pentyl and hexyl.

The term "lower alkoxy" as a moiety in lower-alkoxy-phenyl refers to the group lower-alkyl-O-phenyl wherein lower alkyl is as defined above.

The term "halo" as a radical denotes fluoro, chloro, bromo and iodo.

The term "functional derivative" of the carboxy includes esters and amides which are used as protective group for carboxy group. Examples of the esters are aliphatic esters, for example, C_{1-6} alkyl ester such as methyl ester, ethyl ester, propyl ester, isopropyl ester, butyl ester, isobutyl ester, t-butyl ester, pentyl ester, 1-cyclopropylethyl ester, etc., lower alkenyl ester such as vinyl ester, allyl ester, etc., lower alkynyl ester such as ethynyl ester, propynyl ester, etc., lower alkoxy(lower)-alkyl ester such as methoxymethyl ester, 1-methoxyethyl ester, etc., and aromatic esters, for example, optionally substituted aryl ester such as phenyl ester, tolyl ester, t-butylphenyl ester, salicyl ester, 3,4-di- methoxyphenyl, etc., aryl(lower)alkyl ester such as benzyl ester, trityl ester, benzhydryl ester ester, etc. Examples of the amides are lower alkyl amides such as methylamide, ethylamide, etc., and lower alkylsulfonylamide such as methanesulfonylamide, ethanesulfonylamide, etc.

The term "saturated or unsaturated lower aliphatic hydrocarbon residue" include straight or branched chain aliphatic hydrocarbon residue which may have at least one double or triple bond and up to 6 carbon atoms in the principal chain and up to 3 carbon atoms in any side chain. Such residue may be partly or completely cyclic. Examples of preferred residues for R_1 are $-(CH_2)_4-$, $-(CH_2)_2CH=CH-$, $-(CH_2)_2CH(CH_3)CH_2-$, $=CH(CH_2)_3-$, $-(CH_2)_2-CH=CH-$,

$-CH=CH-(CH_2)_4$, etc., and examples of preferred residues for R_2 are $-(CH_2)_5-$, $-(CH_2)_6-$, $-(CH_2)_7-$, $-(CH_2)_8-$, $-(CH_2)_9-$, $-(CH_2)_3CH(CH_3)CH_2-$, $-C(CH_3)_2(CH_2)_4-$, $-CH_2CH(CH_3)(CH_2)_4-$, $-CH_2CH(CH_3)(CH_2)_5-$, $-C(CH_3)(OCH_3)(CH_2)_4-$, $-C(CH_3)_2CH_2O(CH_2)_2$, $-C(CH_3)_2CH_2O(CH_2)_3-$, cyclopentyl, cyclohexyl, $-CH_2CH(CH_3)(CH_2)_2CH=CH(CH_3)CH_2-$, $-CH(CH_3)CH_2C\equiv CCH_2-$, phenyl, phenoxy, etc.

The configration of the ring and α- and/or omega chain in the above formula (I) may be the same as or different from that in the natural prostaglandins. However, the present invention also include a mixture of a compound having natural configuration and that of unnatural configuration.

Suitable "pharmaceutically acceptable salt" includes conventional non-toxic salt, and may be a salt with an inorganic base, for example a metal salt such as an alkali metal salt (e.g. sodium salt, potassium salt, etc.) and an alkaline earth metal salt (e.g. calcium salt, magnesium salt, etc.), ammonium salt, a salt with an organic base, for example, an amine salt (e.g. trimethylamine salt, triethylamine salt, cyclohexylamine salt, benzylamine salt, ethylenediamine salt, ethanolamine salt, diethanolamine salt, triethanolamine salt, tris(hydroxymethylamino)methane salt, procaine salt, caffeine salt, etc.), a basic amino acid salt (e.g. arginine salt, lysine salt, etc.) and the like. These salts can be prepared by the conventional process, for example from the corresponding acid and base or

by salt interchange.

The above compounds of the formula (I) include, for example,

13,14-dihydro-15-keto-PGA_1s, 13,14-dihydro-15-keto-PGA_2s, 13,14-dihydro-15-keto-PGA_3s, 13,14-dihydro-15-keto-PGB_1s, 13,14-dihydro-15-keto-PGB_2s, 13,14-dihydro-15-keto-PGB_3s, 13,14-dihydro-15-keto-PGC_1s, 13,14-dihydro-15-keto-PGC_2s, 13,14-dihydro-15-keto-PGC_3s, 13,14-dihydro-15-keto-PGD_1s, 13,14-dihydro-15-keto-PGD_2s, 13,14-dihydro-15-keto-PGD_3s, 13,14-dihydro-15-keto-PGE_1s, 13,14-dihydro-15-keto-PGE_2s, 13,14-dihydro-15-keto-PGE_3s, 13,14-dihydro-15-keto-PGF_1s, 13,14-dihydro-15-keto-PGF_2s, 13,14-dihydro-15-keto-PGF_3s, 13,14-dihydro-15-keto-PGI_1s, 13,14-dihydro-15-keto-PGI_2s, 13,14-dihydro-15-keto-PGI_3s, 13,14-dihydro-15-keto-PGJ_1s, 13,14-dihydro-15-keto-PGJ_2s, and 13,14-dihydro-15-keto-PGJ_3s.

Typical examples of the above compounds of the formula (I) include:

(1) 13,14-dihydro-15-keto-PGA_1 methyl ester

(2) 13,14-dihydro-15-keto-PGA_1 isopropyl ester

(3) 13,14-dihydro-15-keto-PGA_2 methyl ester

(4) 13,14-dihydro-15-keto-PGA_2 isopropyl ester

(5) 13,14-dihydro-15-keto-20-ethyl-PGA_1 methyl ester

(6) 13,14-dihydro-15-keto-20-ethyl-PGA_1 isopropyl ester

(7) 13,14-dihydro-15-keto-20-ethyl-PGA_2 methyl ester

(8) 13,14-dihydro-15-keto-20-ethyl-PGA_2 isopropyl ester

(9) 13,14-dihydro-15-keto-PGA_2

(10) 13,14-dihydro-15-keto-PGB_1 methyl ester

(11) 13,14-dihydro-15-keto-PGB_1 isopropyl ester

(12) 13,14-dihydro-15-keto-PGB_2 methyl ester

(13) 13,14-dihydro-15-keto-PGB_2 isopropyl ester

(14) 13,14-dihydro-15-keto-20-ethyl-PGB_1 methyl ester

(15) 13,14-dihydro-15-keto-20-ethyl-PGB_1 isopropyl ester

(16) 13,14-dihydro-15-keto-20-ethyl-PGB_2 methyl ester

(17) 13,14-dihydro-15-keto-20-ethyl-PGB_2 isopropyl ester

(18) 13,14-dihydro-15-keto-PGB_2

(19) 13,14-dihydro-15-keto-PGC_1 methyl ester

(20) 13,14-dihydro-15-keto-PGC_1 isopropyl ester

(21) 13,14-dihydro-15-keto-PGC_2 methyl ester

(22) 13,14-dihydro-15-keto-PGC_2 isopropyl ester

(23) 13,14-dihydro-15-keto-20-ethyl-PGC_1 methyl ester

(24) 13,14-dihydro-15-keto-20-ethyl-PGC_1 isopropyl ester

(25) 13,14-dihydro-15-keto-20-ethyl-PGC_2 methyl ester

(26) 13,14-dihydro-15-keto-20-ethyl-PGC_2 isopropyl ester

(27) 13,14-dihydro-15-keto-PGC_2

(28) 13,14-dihydro-15-keto-PGD_1 methyl ester

(29) 13,14-dihydro-15-keto-PGD_1 ethyl ester

(30) 13,14-dihydro-15-keto-PGD_2 ethyl ester

(31) 13,14-dihydro-15-keto-PGD_2 buthyl ester

(32) 13,14-dihydro-15-keto-5,6-dehydro-$PGDA_2$ methyl ester

(33) 13,14-dihydro-15-keto-5,6-dehydro-9 -hydroxy-PGD_2

(34) 13,14-dihydro-15-keto-5,6-dehydro-9 -hydroxy-PGD_2 methyl ester

(35) 13,14-dihydro-15-keto-16R,S-fluoro-PGD_2 methyl ester

(36) 13,14-dihydro-15-keto-16,16-dimethyl-PGD_2 methyl ester

(37) 13,14-dihydro-15-keto-19-methyl-PGD_2 methyl ester

(38) 13,14-dihydro-15-keto-20-methoxy-PGD_2

(39) 13,14-dihydro-15-keto-20-methoxy-PGD_2 butyl ester

(40) 13,14-dihydro-15-keto-16R,S-methyl-20-methoxy-PGD_2 methyl ester

(41) 13,14-dihydro-15-keto-20-ethyl-PGD_1 methyl ester

(42) 13,14-dihydro-15-keto-20-ethyl-PGD_1 ethyl ester

(43) 13,14-dihydro-15-keto-20-ethyl-PGD_2 methyl ester

(44) 13,14-dihydro-15-keto-20-ethyl-PGD_2 ethyl ester

(45) 13,14-dihydro-15-keto-20-methoxyethyl-PGD_2 methyl ester

(46) 13,14-dihydro-15-keto-PGD_2

(47) 13,14-dihydro-15-keto-PGE_1 ethyl ester

(48) 13,14-dihydro-6,15-diketo-PGE_1 ethyl ester

(49) 13,14-dihydro-6,15-diketo-PGE_1 butyl ester

(50) ±13,14-dihydro-6,15-diketo-PGE_1 ethyl ester

(51) 13,14-dihydro-6,15-diketo-11-dehydroxy-11R-methyl-PGE_1 ethyl ester

(52) 13,14-dihydro-6,15-diketo-16R,S-fluoro-11-dehydroxy-11R-methyl-PGE_1 ethyl ester

(53) 13,14-dihydro-6,15-diketo-16,16-dimethyl-PGE_1 ethyl ester

(54) 13,14-dihydro-6,15-diketo-19-methyl-PGE_1 methyl ester

(55) 13,14-dihydro-6,15-diketo-11-dehydroxy-11R-hydroxymethyl-PGE_1 ethyl ester

(56) 13,14-dihydro-15-keto-PGE_2

(57) 13,14-dihydro-15-keto-PGE_2 methyl ester

(58) 13,14-dihydro-15-keto-PGE_2 isopropyl ester

(59) 13,14-dihydro-15-keto-Δ^2-PGE_2 methyl ester

(60) 13,14-dihydro-15-keto-16R,S-fluoro-PGE_2 ethyl ester

(61) 13,14-dihydro-15-keto-3,16-dimethyl-PGE_2 methyl ester

(62) 13,14-dihydro-15-keto-16R,S-hydroxy-PGE_2 ethyl ester

(63) 13,14-dihydro-15-keto-19-methyl-PGE_2 ethyl ester

(64) 13,14-dihydro-15-keto-20-methoxy-PGE_2 methyl ester

(65) 13,14-dihydro-15-keto-20-methoxy-Δ^2-PGE_2 methyl ester

(66) 13,14-dihydro-15-keto-16,16-dimethyl-20-methoxy-PGE_2 methyl ester

(67) 13,14-dihydro-15-keto-20-ethyl-PGE_1 methyl ester

(68) 13,14-dihydro-6,15-diketo-20-ethyl-PGE_1 ethyl ester

(69) 13,14-dihydro-6,15-diketo-20-ethyl-PGE_1 methyl ester

(70) 13,14-dihydro-15-keto-20-ethyl-PGE_2 methyl ester

(71) 13,14-dihydro-15-keto-20-ethyl-PGE_2 ethyl ester

(72) 13,14-dihydro-15-keto-20-n-propyl-PGE_2 methyl ester

(73) 13,14-dihydro-15-keto-20-ethyl-11-dehydroxy-11R-methyl-PGE_2 methyl ester

(74) 13,14-dihydro-15-keto-PGE_2

(75) 13,14-dihydro-15-keto-$PGF_{1\alpha}$ ethyl ester

(76) 13,14-dihydro-15-keto-$PGF_{2\alpha}$ methyl ester

(77) 13,14-dihydro-15-keto-$PGF_{2\alpha}$ ethyl ester

(78) 13,14-dihydro-15-keto-9 -hydroxy-$PGF_{2\alpha}$ methyl ester

(79) 13,14-dihydro-15-keto-16R,S-fluoro-$PGF_{1\alpha}$

(80) 13,14-dihydro-15-keto-16R,S-fluoro-$PGF_{2\alpha}$

(81) 13,14-dihydro-15-keto-16R,S-fluoro-11-dehydroxy-11R-methyl-$PGF_{2\alpha}$ methyl ester

(82) 13,14-dihydro-15-keto-16,16-dimethyl-$PGF_{2\alpha}$ ethyl ester

(83) 13,14-dihydro-15-keto-17S-methyl-$PGF_{2\alpha}$ ethyl ester

(84) 13,14-dihydro-15-keto-20-ethyl-$PGF_{1\alpha}$ methyl ester

(85) 13,14-dihydro-15-keto-20-ethyl-$PGF_{2\alpha}$

(86) 13,14-dihydro-15-keto-20-ethyl-$PGF_{2\alpha}$ methyl ester

(87) 13,14-dihydro-15-keto-20-ethyl-$PGF_{2\alpha}$ ethyl ester

(88) 13,14-dihydro-15-keto-20-ethyl-$PGF_{2\alpha}$ isopropyl ester

(89) 13,14-dihydro-15-keto-20-ethyl-$PGF_{2\alpha}$ butyl ester

(90) 13,14-dihydro-15-keto-20-methyl-$PGF_{2\alpha}$ methyl ester

(91) 13,14-dihydro-15-keto-20-n-propyl-$PGF_{2\alpha}$ methyl ester

(92) 13,14-dihydro-15-keto-20-n-butyl-$PGF_{2\alpha}$ methyl ester

(93) 13,14-dihydro-15-keto-20-ethyl-16R,S-fluoro-$PGF_{2\alpha}$

(94) 13,14-dihydro-15-keto-20-ethyl-16R,S-fluoro-$PGF_{2\alpha}$ methyl ester

(95) 13,14-dihydro-15-keto-20-ethyl-11-dehydroxy-11R-methyl-$PGF_{2\alpha}$ methyl ester

(96) 13,14-dihydro-15-keto-20-ethyl-16R,S-fluoro-11-dehydroxy-11R-methyl-$PGF_{2\alpha}$ methyl ester

(97) 13, 14-dihydro-15-keto-$PGF_{2\alpha}$

Examples of substituents present in preferred compounds are as follows: Substituents on the carbon atom at 3-, 17- and/or 19-position include lower alkyl, for example,

C_{1-4} alkyl, especially methyl and ethyl. Substituents on the carbon atom at 16-position include lower alkyl such as methyl, ethyl etc., hydroxy and halogen atom such as chlorine, fluorine etc. Substituents on the carbon atom at 20-position include saturated and unsaturated lower alkyl such as C_{1-4} alkyl, lower alkoxy such as C_{1-4} alkoxy and lower alkoxy (lower) alkyl such as C_{1-4} alkoxy-C_{1-4} alkyl. Prostaglandins having hydroxy substituent on the carbon atom at 9- and/or 11-position include PGDs, PGEs and PGFs, stereochemistry of which at 9- and/or 11-carbon atom being alpha, beta or a mixture thereof.

Within the above exemplified compounds, 13,14-dihydro-15-keto-PGDs can be prepared according to the process described in EP0281239A1, 13,14-dihydro-15-keto-PGEs in EP0284180A1 and 13,14-dihydro-15-keto-PGFs in EP0289349 or European Patent Application No. 88308299.2, and other compounds can be prepared referring to the above processes.

The compounds of the formula(I) wherein Z is a derivative of carboxy group can be prepared by reacting the corresponding free carboxylic acid or a reactive derivative thereof with an alcohol, amine or a reactive derivative at the hydroxy or amino group thereof. The reactive derivatives at the carboxy group include acid halides, acid anhydrides, activated esters and activated amides. As the acid halides, acid chloride is most commonly used. The acid anhydrides include symmetiric anhydride and mixed anhydride. Examples of the latter are dialkyl phosphoric acid mixed

anhydride, dialkyl phosphorous acid mixed anhydride, alkyl carbonic acid mixed anhydride and aliphatic acid (e.g. pivalic acid, trichloroacetic acid, etc.) mixed anhydride. The activated ester include aliphatic ester such as methyl ester, ethyl ester and cyanomethyl ester, aromatic ester such as p-nitrophenyl ester, esters with N-hydroxy compounds such as N-hydroxysuccinimide. The activated amides include amides with imidazole, dimethylimidazole and triazole. The reactive derivatives at the hydroxy group include halide and sulfonic acid (e.g. methanesulfonic acid, toluenesulfonic acid etc.). The reactive derivatives at the amino group include Schiff's base with aldehydes (e.g. acetaldehyde, isopentanal, benzaldehyde etc.), reaction products with silyl compounds (e.g. trimethylsilyl chloride, trimethylsilyl acetamide etc.) and reactive products with phosphorus compounds (e.g. phosphorus trichloride, phosphorus oxychloride etc.).

When a free carboxylic acid is to be used, the reaction is advantageously carried out in the presence of a condensing agent. Examples of the condensing agents are N,N-dicyclohexylcarbodimide, N-cyclohexyl-N'--morpholinoethylcarbodiimide, N,N'-diisopropylcarbodiimide, N-ethylbenzisoxazolium salt, 2-chloro-1-methylpyridinium salt, N,N'-carbonyldiimidazole, phosphorus trichloride, phosphorus oxychloride etc. The reaction is usually carried out in a solvent. Examples of the solvents are dioxane, methylene chloride, tetrahydrofuran, dimethylformamide,

pyridine, benzene, toluene, xylene etc.

The etherified cyclodextrins used according to the present invention include lower-alkylated cyclodextrins such as methyl cyclodextrin, ethylcyclodextrin, propylcyclodextrin, dimethylcyclodextrin, trimethylcyclodextrin etc., lower-alkenylated cyclodextrins, hydroxy--lower-alkylated cyclodextrins such as hydroxyethylcyclodextrin, hydroxypropylcyclodextrin etc., lower-alkoxy--lower-alkylated cyclodextrines, aralkylated cyclodextrins, such as benzylcyclodextrin etc., halo-lower-alkylated cyclodextrins such as chloroethylcyclodextrin etc., and cyclodextrin-epichlorohydrin-copolymers. These are known or can be prepared by a process analogous to that for the known compounds.

The term "intimate mixture" used herein refers to a mixture wherein two or more substances are minutely mixed and dispersed as in a solid solution and wherein constituent substances cannot be visually distinguished. It is to be appreciated that the intimate mixture include a mixture wherein the mixed constituents form an adduct or inclusion compound.

Said intimate mixture can be prepared, for example, by contacting a 13,14-dihydro-15-keto-PGs with an etherified cyclodextrine in a solvent or a solvent system in which at least one of them is at least partyl soluble and then removing said solvent or solvent system. Preferred

solvents or solvent systems are those in which both the constituents are completely soluble. Examples of preferred solvents and solvent systems are water and hydrophilic organic solvents such as methanol, ethanol, dioxane, acetone etc. as well as a mixture thereof. Dissolution can be carried out at or below room temperature or with moderate heating. The removal of the solvent can preferablely be effected by dislillation under reduced pressure or lyophilization. Resulting residue can conveniently be pulverized. The etherized cyclodextrin is suitably used in an amount more than 5 times, preferably 5 or 7 to 200 times and more preferably 5 or 8 to 100 times the amount of the 13,14-dihydro-15-keto-PGs.

The thus obtained powders can be converted into conventional solid or liquid preparations which are suitable for peroral or parenteral administration (e.g. intravenou. intraarterial, subcutaneous, intramuscular, intravaginal, intrauterine etc.) such as tablets, powders, granules, readily soluble solid, suspension etc. The preparation may be prepared by conventional process using carriers or diluents such as starch, lactose, dextrin, mannitol water, and lubricant, humectant, perfume, preservative, colorant etc. Aqueous solution may be prepared by directly dissolving the two ingredients and without passing through the powders.

For the above preparations, the amount contained therein of the 13,14-dihydro-15-keto-PGs will of course vary

depending the treatment desired in obestetic-gynecologic, cardiovascular, gastromtestinal, ophthalmologic regions etc., and further on age, condition, severity of patient. In general, satisfactory results are obtainable in administration at a dosage of 0.005 - 500 mg.

A more complete understanding of the present invention can be obtained by reference to the following Examples which are provided herein for purposes of illustration only, and are not intended to limit the scope of the invention.

The following abbreviations are used in Examples.

CD: Cyclodextrine

Compound A: 13,14-Dihydro-15-keto-PGE_2 ethyl ester

Compound B: 13,14-Dihydro-15-keto-19-methyl-PGE_2 ethyl ester

Compound C: 13,14-Dihydro-6,15-diketo-PGE_1 ethyl ester

Compound D: 13,14-Dihydro-6,15-diketo-19-methyl-PGE_1 ethyl ester

Compound E: 13,14-Dihydro-6,15-diketo-11--dehydroxy-11R-methyl-PGE_1 ethyl ester

Compound F: 13,14-Dihydro-15-keto-16R,S-fluoro-PGE_2

Compound G: 13,14-Dihydro-15-keto-20-ethyl-$PGF_{2\alpha}$ isopropyl ester

Compound H: 13,14-Dihydro-15-keto-16R,S-fluoro-$PGF_{2\alpha}$ methyl ester

Example 1

(Dimethyl-α-CD: Compound D=40:1)

An aliquot (4ml) of an aqueous solution (Solution A) containing 100mg/ml of dimethy-α-CD was added to 10mg of compound D. The resulted mixture was stirred at room temperature until it was homogeneous and lyophilized to give a white solid in which both the components were intimately mixed.

Comparative Example 1

(α-CD: Compound D=40:1)

An aliquot (4ml) of an aqueous solution (Solution X) containing 100mg/ml of α-CD was added to 10mg of compound D. The resulted mixture was stirred at room temperature but did not give a homogeneous solution and formed distinguishable solid and liquid phases.

Example 2

(Dimethyl-α-CD: Compound D=20:1)

The procedure in Example 1 was repeated using 2ml in place of 4ml of Solution A. An analogous white solid was obtained.

Comparative Example 2

(α-CD: Compound D=20:1)

The procedure in Comparative Example 1 was repeated using 2ml in place of 4ml of Solution X. An analogous result was obtained.

Example 3

(Dimethyl-α-CD: Compound D=10:1)

The procedure in Example 1 was repeated using 1ml in place of 4ml of Solution A. An analogous white solid was obtained.

Comparative Example 3

(α-CD: Compound D=10:1)

The procedure in Comparative Example 1 was repeated using 1ml in place of 4ml of Solution X. An analogous result was obtained.

Example 4

(Dimethyl-β-CD: Compound D=20:1)

The procedure in Example 2 was repeated using a solution (Solution B) containing 100mg/ml of dimethyl-β-CD in place of Solution A. An analogous white solid was obtained.

Example 5

(Dimethyl-β-CD: Compound D=10:1)

The procedure in Example 4 was repeated using 1ml in place of 2ml of Solution B. An analogous white solid was obtained.

Comparative Example 4

(β-CD: Compound D=13.5:1)

The procedure in Comparative Example 1 was repeated using 10ml of a solution (Solution Y) containing 13.5mg/ml of β-CD in place of Solution X. An analogous result was obtained.

Comparative Example 5

(γ-CD: Compound D=30:1)

The procedure in Comparative Example 1 was repeated using 1.25ml of a solution (Solution Z) containing 240mg/ml of γ-CD. An analogous result was obtained.

Example 6

(Hydroxypropyl-β-CD: Compound D=40:1)

The procedure in Example 1 was repeated using 4ml of solution (Solution C) containing 100mg/ml of hydroxypropyl-β-CD in place of Solution A. An analogous white solid was obtained.

Example 7

(Dimethyl-α-CD: Compound D=30:1)

The procedure in Example 1 was repeated using 3ml of Solution A and a compound selected from Compound A, B, C, E and F. An analogous white solid was obtained.

Comparative Example 6

(α-CD: Compound D=30:1)

The procedure in Comparative Example 1 was repeated using 3ml of Solution X and a compound selected from Compound A, B, C, E and F. An analogous result was obtained.

Comparative Example 7 (Known Technique)

(α-CD: PGE_2=30:1)

The procedure in Comparative Example 1 was repeated using 3ml of Solution X and 10mg of PGE_2 to give a

homogeneous solution, which, on lyophilization, gave a white solid.

Example 8

(Dimethyl-α-CD: Compound G=20:1)

The procedure in Example 1 was repeated using 2ml of Solution A and Compound G. An analogous white solid was obtained.

Example 9

(Heat Stability)

Each of the white solids obtained in Examples 1-5 was tested for heat stability by heating in a thermostatic oven held at 60°C. Residual rate of Compound D are shown in Table 1.

Assay protocol for residual rate was as follows: The sample heated for a predetermined period of time was dissolved in aliquot of distilled water. A prescribed amount of the solution was assayed by High Performance Liquid Chromatography (HPLC) (Instrument: Hitachi 655A) under the following conditions.

Detection Wavelength 210nm

Mobile Phase $CH_3CN:H_2O$=1:1

Flow Rate 1ml/min

Column Package High Pore RP-18,

Particle Size: 10μm, 4x250mm

Table 1

Sample	Residual Rate of Compound D(%)	
	60°C, 5 Days	60°C, 14 Days
Example 1	100	99
" 2	100	99
" 3	100	99
" 4	99	98
" 5	98	96
Compound D (as such)	86	56

The above results demonstrate that the stability of Compound D was improved by etherized cyclodextrins.

Example 10

(Stability in Aqueous Solution)

Each of the white solids obtained in Examples 3 and 5 was dissolved in distilled water to form a solution containing 44mg/ml (4mg Compound D/ml). The solution was tested for stability by placing in a stoppered vial for a predetermined period of time at room temperature. Residual rate of Compound D are shown in Table 2.

Assay protocal for residual rate using internal standard HPLC was as follows:

The sample (aqueous solution) left for a predetermined period of time at room temperature was

combined with an acetonitrile solution containing an amount of internal standard. After mixing thoroughly, an aliquot of the solution was assayed by HPLC (instrument:Hitachi 655A) under the following conditions.

Detection Wavelength 282nm

Mobile Phase $CH_3CN:H_2O=3:2$

Flow Rate 1ml/min

Column Package High Pore RP-18,

Particle Size: 10μm,

4x250mm

Table 2

Sample	Residual Rate of Compound D(%)			
	Room Temperature, Days			
	1	2	3	4
Example 3	98.4	96.8	95.9	89.2
" 5	99.5	96.6	96.0	91.3

The above results demonstrate that the stability of Compound D was improved by etherized cyclodextrins.

Example 11

(Dimethyl-α-CD: Compound H = 8:1)

The procedure in Example 1 was repeated using 4 ml of Solution A and Compound H. An analogous white solid was obtained.

Comparative Example 8

(α-CD: Compound H = 8:1)

The procedure in Comparative Example 1 was repeated using 4 ml of Solution X and Compound H. An analogous result was obtained.

Example 12

(Heat Stability and Stability in Aqueous Solution)

The tests for the heat stability and the stability in the aqueous solution were carried out according to the procedures described in Examples 9 and 10 using the white solid obtained in Example 11. The results are shown in Tables 3 and 4.

Assay protocal for residual rate of Compound H was as follows:

The sample heated for a predetermined period of the time was dissolved in an aliquot of acetonitrile solution and an aliquot of the solution was assayed by HPLC. For the aqueous solution, the solution left for a predetermined period of time at room temperature was directly assayed by HPLC. In both cases, HPLC was performed using Hitachi 655A under the following conditions.

Detection Wavelength 200nm

Mobile Phase $CH_3CN:H_2O=3:2$

Flow Rate 1ml/min

Column Package:Lichro-CART RP-18(Merck)

Particle Size: 10μm

4x250mm

Table 3

Sample	Residual Rate of Compound H(%)	
	60°C, 5days	60°C, 14days

Table 4

Sample	Residual Rate of Compound H(%)	
	RT, 1day	RT, 2days
Example 9	100	100

Example 13

(Heat Stability)

The white solid obtained in Example 3 was tested for heat stability by placing in a thermostat for a predetermined period of time at 60, 70 or 80°C. The results are shown in Table 5.

Assay protocal for residual rate using internal standard HPLC was as follows:

The sample heated for a predetermined period of time was combined with an acetonitrile solution containing an amount of internal standard. After mixing thoroughly, an aliquot of the solution was assayed by HPLC (instrument: Hitachi 655A) under the following conditions.

Detection Wavelength 200nm

Mobile Phase $CH_3CN:H_2O$=3:2

Flow Rate 1ml/min

Column Package:Lichro-CART RP-18(Merck)

Particle Size: 10μm

4x250mm

Table 5

Sample	Residual Rate of Compound D(%)		
	60°C, 5days	70°C, 28days	80°C, 12days
Example 3	95.6	95.9	93.9

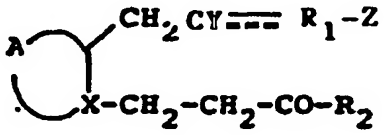
WHAT I/WE CLAIM IS:
~~What is claimed is:~~

1. A stabilized 13,14-dihydro-15-ketoprostaglandin composition comprising an intimate mixture of

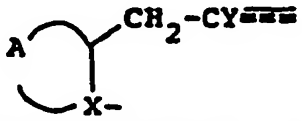
a) a therapeutically effective amount of at least one compound selected from 13,14-dihydro-15-ketoprostaglandins and

b) at least one compound selected from pharmaceutically acceptable etherized cyclodextrins.

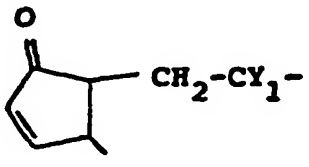
2. The composition of claim 1, in which said 13,14-dihydro-15-ketoprostaglandins are represented by the formula:



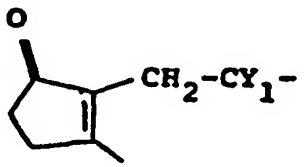
(I)

wherein the group: 

is a radical selected from the group consisting of the following formulae:



(i)



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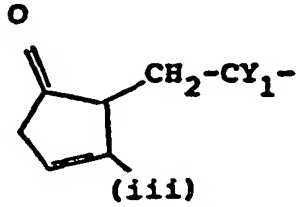

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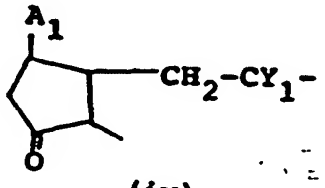

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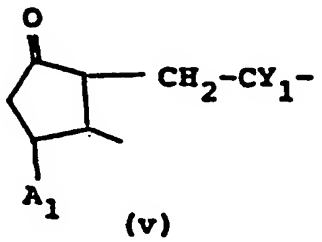

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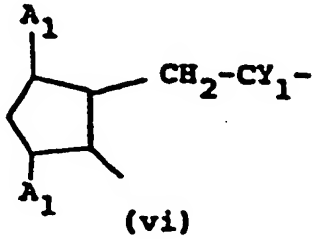

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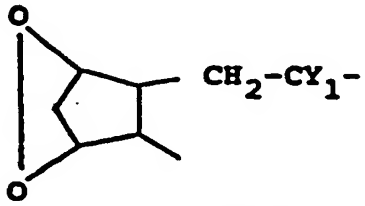

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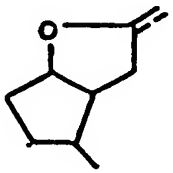

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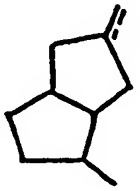
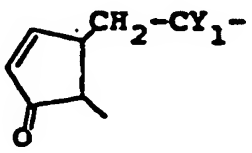
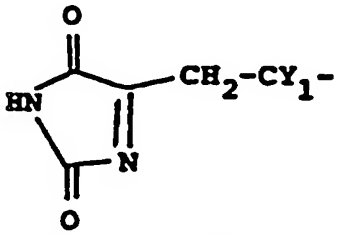


(ix)



(x)



(xi)

A_1 is hydroxy, lower alkyl or hydroxy(lower)alkyl,

Y_1 is 0, 1 or 2 hydrogen atoms or oxo,

Z is hydroxymethylcarbonyl, carboxy or a functional derivative of carboxy,

R_1 is saturated or unsaturated lower aliphatic hydrocarbon residue,

R_2 is saturated or unsaturated lower aliphatic hydrocarbon residue which is unsubstituted or substituted with at least one substituent selected from the group consisting of hydroxy, halo, lower-alkoxyphenyl and phenoxy,

the symbol of a line and a dotted line is single bond or double bond, and

the symbol of a line and two dotted line is single bond, double bond or triple bond,

or a pharmaceutically acceptable salt thereof.

3. The composition of claim 1, in which said etherized cyclodextrins are cyclodextrin(lower)alkyl ethers, cyclodextrin(lower)alkenyl ethers, cyclodextrin-hydroxy-(lower)alkyl ethers, cyclodextrin(lower)alkoxy(lower)alkyl ethers, cyclodextrin-monocyclic aromatic(lower)alkyl ethers, cyclodextrin-halo(lower)alkyl ethers, or cyclodextrin-epichlorhydrin-copolymers.

4. The composition of claim 1, in which the ratio by weight of component a) to component b) is between 1:5 and 1:200.

5. The composition of claim 4, in which the ratio is between 1:7 and 1:200.

6. The composition of claim 4, in which the ratio is between 1:5 and 1:100.

7. The composition of claim 4, in which the ratio is between 1:8 and 1:100.

8. The composition of claim 4, in which the ratio is between 1:10 and 1:40.

9. A method of preparing a stabilized 13,14-dihydro-15-ketoprostaglandin composition which comprises intimately mixing

a) a therapeutically effective amount of at least one compound selected from 13,14-dihydro-15-ketoprostaglandins and

b) at least one compound selected from pharmaceutically acceptable etherized cyclodextrins.

10. A method of stablizing 13,14-dihydro-15-ketoprostaglandins which comprises contacting (A) 13,14-dihydro-15-ketoprostaglandin with (B) etherized cyclodextrin in a solvent capable of at least partly dissolving at least one of (A) and (B).

11. A composition according to claim 1 substantially as herein described or exemplified.

12. A method according to claim 9 or 10 substantially as herein described or exemplified.

KABUSHIKIKAISHA UENO SEIYAKU OYO KENKYUJO
By Their Attorneys
HENRY HUGHES LIMITED
By: [signature]

END